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RESEARCH ARTICLE

NOVEL 2-OXO-THIADIAZOLE-1, 2, 3, 4-TETRAHYDROPYRIMIDINE DERIVATIVES SYNTHESIZED BY BIGINELLI REACTION - BIOLOGICAL ACTIVITY AND DOCKING STUDIES

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ABSTRACT

A new series of novel 2-oxo-6-(5-methyl-[1,3,4]-thiadiazol-2-yl sulfanylmethyl)-1, 2, 3, 4-tetrahydropyrimidine derivatives (3a-h) were synthesized by Biginelli reaction and characterized by elemental, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analysis . All the compounds were screened for their in vitro antibacterial activity (*B.Subtilis, Staphylococcus aereus*, *E.coli, K.pneumonia*) and antifungal activity (*C.albicans, A.niger*) by disc diffussion method. Among the tested compounds showed the significant antimicrobial activity. The newly synthesized compounds were docked with glucosamine-6-phosphate synthase enzyme in order to study the accepted binding mode of the active compounds.

INTRODUCTION

Antimicrobial resistance is an evolving predicament in treating the patients, and cause several deaths every year (Onemu *et al*., 2013; Howard *et al*., 1996). The main cause of microbial resistance is the mutations or transfer of resistant genes between organisms. So, we need to develop the novel antimicrobial agents with difficult mechanism of action. Nitrogen based heterocyclic compounds are very abundant in nature since they are present as structural subunits in many natural products such as vitamins, hormones and alkaloids. These compounds are also interesting from an industrial point of view especially for the synthesis of pharmaceuticals, herbicides, pesticides, dyes (Dax *et al*., 1999; Oliver *et al*., 2000; Heys *et al*., 2000; Lu *et al*., 2000). Nitrogen and sulfur heterocyclic system families are very interesting due to their versatile pharmacological activities (Benbrook *et al*., 2002).
Multi-component reactions can provide products with diversity needed in the discovery of new compounds using simple and non hazardous process (Schreiber *et al*., 2000; Prashantha kumar *et al*., 2009; Xingwen *et al*., 2007). A new series of 2-oxo-6-(5-methyl-[1, 3,4]-thiadiazol-2-yl sulfanylmethyl)-1, 2, 3, 4-tetrahydropyrimidine derivatives (3a-h) were synthesized via Biginelli reaction (Suresh *et al*., 2012).

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One prominent multi component reaction that produces an interesting class of nitrogen heterocycles is the venerable Biginelli dihydropyrimidine synthesis. It was synthesized for the first time by Pietro Biginelli in year 1891. It involves the simple one-pot condensation reaction of an aromatic aldehyde, urea and ethylacetoacetate in ethanolic solution (Mohamed *et al*., 2009; Biginelli *et al*., 1893; Yonghong *et al*., 2015; Ali *et al*., 2015; Fabio *et al*., 2001; Mohammad Haji *et al*., 2016; Mohammad Hosein Farjam *et al*., 2016). Bigenelli reaction is a useful three component reaction offering versatile protocol for the production of 1,2,3,4-tetrahydrpyrimidine-2-ones nucleus represented a very important field in drug discovery (Jovana *et al*., 2016). 1,2,3,4-tetrahydrpyrimidine-2-ones and their sulfur analogs have attracted considerable interest because of their wide range of biological activities such as antioxidant (Lakshmi *et al*., 2014), antimalarial (Vivekanand *et al*., 2012), anti-HIV (Vivekanand *et al*., 2012), anticancer(Azza *et al.,* 2012), antibacterial (Haitham *et al*., 2012 ; Shah *et al*., 2009), anti-TB (Tarunkumar *et al*., 2011), anti-inflammatory (Ajitha *et al*., 2011), calcium channel blocker (Hiren *et al*., 2011), antihypertensive(Hiren *et al*., 2011), anti-convulsant (Prabhat *et al*., 2015). We envisaged that presence of sulphur with heterocyclic compund attached in position 6 of the 2-oxo-1, 2, 3,4-tetrahydropyrimidine ring could have an important impact on the biological activity of these molecules. The synthesized compounds bearing sulphur with heterocyclic compound attached in position 6 of the ring

having promising biological activity. We decided to develop synthetic methodologies for sulphur with heterocyclic compound of 2-oxo-1,2,3,4-tetrahydropyrimidine compounds and the results of our studies are reported. Antimicrobial properties of sulphur with heterocyclic compound of 2-oxo-1,2,3,4-tetrahydropyrimidine derivatives have been studied and the results are presented in this study. The structures of the synthesized compounds were assigned based on elemental, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. All newly synthesized compounds were screened for their *in vitro* antimicrobial activity. Molecular docking (Vijesh *et al*., 2013) is very popular method introduced to investigate molecular association and is particularly useful in the drug discovery field to study the binding of small molecules (ligands) to macromolecules (receptor). Docking is frequently used to predict the binding orientation of small drugs candidates to their protein targets in order to in turn predict the affinity and activity of a small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been direct docking.

MATERIALS AND METHODS

Melting points were determined on a Buchi melting point B-540 instrument and are uncorrected. The purity of compounds was analyzed by thin layer chromatography (pre- coated silica gel, Merck). The mass spectra were recorded in PE-SCIEX API-3000 LC/MS/MS with Turbo ion spray. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400MHz Spectrometer with multinuclear BBO probe and TMS as an internal standard. Elemental analyses were performed on a Vario-EL III instrument. The titled 4-phenyl-6-(5-methyl-[1, 3,4] thiadiazol-2-ylsulfanylmethyl)-2-oxo-1, 2, 3, 4-tetrahydro -pyrimidine-5-carboxylic acid ethyl ester 3(a-h) was synthesized by a reported procedure (Mohamed *et al*., 2009 ; Biginelli *et al*., 1893 ; Yonghong *et al*., 2015 ; Ali *et al*., 2015 ; Fabio *et al*., 2001 ; Mohammad Haji *et al*., 2016 ; Mohammad Hosein Farjam *et al*., 2016).

Experimental

Procedure for the synthesis of 4-(5-Methyl-[1, 3,4] thiadiazol-2-ylsulfanyl)-3-oxo-butyric acid ethyl ester (2): Anhydrous potassium carbonate (113.4 mmol) was added to a solution of 5-methyl-[1, 3,4] thiadiazole-2-thiol (**1**) (75.6 mmol) in dimethylformamide (25 mL). To the reaction mixture, ethyl-4-chloroacetocetate (83.1 mmol) was added slowly at room temperature under stirring. The progress of the reaction was monitored by thin layer chromatography using a mixture of ethyl acetate and n-hexane (3:7) as eluent. The by-product potassium chloride was removed by filtration. The mother liquor containing the product was concentrated under vacuum to remove dimethyl formamide and the residual dimethyl formamide was removed using methanol to afford pale brown liquid of 4-(5-Methyl-[1, 3, 4] thiadiazol-2-ylsulfanyl)-3-oxo-butyric acid ethyl ester (**2**) MS: *m/z* 260 (M+), 261 (MH +) was used for next step.

General procedure for the synthesis of 4-phenyl-6-(5-methyl-[1,3,4]thiadiazol-2-ylsulfanylmethyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester 3(a-h): A mixture of 4-(5-Methyl-[1,3,4]thiadiazol-2-ylsulfanyl)-3-oxo-butyric acid ethyl ester (2) (1.9 mmol), arylaldehyde (1.9 mmol) and urea (2.8 mmol) in the presence of concentrated hydrochloric acid (4 drops) in ethanol (5 mL) was heated under reflux till completion of reaction. The reaction was monitored by thin layer chromatography using mixture of chloroform and methanol (9:1) as eluent. The reaction mass was cooled to 30°C and quenched into water to crystallize the product. On filtration and washing with water followed by re-crystallization from ethanol (5 mL): hexane (5 mL) afforded 4-phenyl-6-(5-methyl-[1,3,4]thiadiazol-2-ylsulfanylmethyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester derivatives 3(a-h).

Ethyl-4-(4-fluorophenyl)-6-{[(5-methyl-1,3,4-thiadiaz ol-2-yl)thio]methyl}-2-oxo-1,2,3,4-tetrahydropyrimid ine-5-carboxylate (3a): Yield (82.1%), w. 0.64g, m.p. 158-160°C pale yellow solid; IR v (cm^{-1}) (KBr): 3364 (N-H), 3105 (C-H), 2968 (methyl C-H), 1688 (C=O), 1301(C-O); ^{1}H NMR (400MHz); (DMSO-d_6): δ 9.33 (s, 1H) NH; 7.86(s, 1H) NH; 7.13-7.27 (m, 4H) ArH; 5.17 (d, 1H, J=4Hz) CH; 4.4-4.54 (dd, 2H) SCH_2; 3.99 (q, 2H, J =8Hz) CH_2; 2.69 (s, 3H) CH_3; 1.06 (t, 3H, J= 8Hz) CH_3 . ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.9, 164.5, 163.3, 160.2, 151.7, 146.2, 140.3, 128.3, 128.2, 115.2, 115.0, 101.6, 59.8, 53.3, 33.1, 15.2, 13.8., MS m/*z*: 408 (M^+), 409 $(M+H)^+$. Anal.calcd. for $C_{17}H_{17}FN_4O_3S_2$; M.wt. 408.47; C, 49.99; H, 4.19; N, 3.72; S, 15.7(%); Found: C, 49.98; H, 4.18; N, 3.71; S, 15.69(%).

Ethyl-4-(2-chlorophenyl)-6-{[(5-methyl-1,3,4thiadiaz ol-2-yl)thio]methyl}-2-oxo-1,2,3,4-tetrahydropyrimid ine-5-carboxylate (3b): Yield (77.5%), w. 0.63g, m.p. 200-202°C pale yellow solid; IR v (cm^{-1}) (KBr): 3223 (N-H) , 3089 (C-H), 2978(methyl C-H), 1699 (C=O), 1296 (C-O); ^{1}H NMR (400MHz); ($CDCl_3$): δ 8.55(s, 1H) NH; 7.20-7.38 (m, 4H)ArH; 5.9 (d, 1H); 5.4 (s, 1H); 4.70-4.87 (dd, 2H)SCH_2; 4.07 (q, 2H, J =4Hz) CH_2; 2.74 (s, 3H) CH_3;1.06 (t, 3H, J = 8Hz) CH_3. ^{13}C NMR (100 MHz); ($CDCl_3$): δ 166.7, 166.0, 165.2, 151.5, 148.7, 139.4, 132.7, 129.9, 129.6, 128.1, 127.8, 100.2, 60.7, 52.2, 31.4, 15.9, 14.1., MS m/*z*: 424 (M^+), 425 $(M+H)^+$. Anal.calcd. for $C_{17}H_{17}ClN_4O_3S_2$; M.wt. 424.92; C, 48.05; H, 4.03; N, 13.19.S, 15.0 (%); Found: C, 48.03; H, 4.04; N, 13.18; S, 14.99(%).

Ethyl-4-(3-hydroxyphenyl)-6-{[(5-methyl-1,3,4thiadia zol-2-yl)thio]methyl}-2-oxo-1,2,3,4tetrahydropyrimid ine-5-carboxylate (3c): Yield (84%), w. 0.65g, m.p. 195-197°C pale yellow solid; IR v (cm^{-1}) (KBr): 3355 (N-H) , 3099 (C-H), 2968 (methyl C-H), 1687 (C=O), 1296 (C-O); 1H NMR (400MHz); (DMSO-d_6): δ 9.39 (s, 1H) NH; 9.30 (s, 1H); 7.81(s, 1H) NH; 6.6-7.0 (m, 4H) ArH; 5.17 (d, 1H, J= 4Hz) CH; 4.4-4.54 (dd, 2H) SCH_2; 3.99 (q, 2H, J= 8Hz) CH_2; 2.69 (s, 3H) CH_3;1.06 (t, 3H, J =8Hz) CH_3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.8, 164.3, 163.6, 151.0, 148.0, 146.4, 146.0, 134.6, 115.4, 114.9, 110.1, 101.2, 59.7, 53.7, 32.9, 15.2, 13.8., MS *m/z*: 406 (M^+), 407 $(M+H)^+$. Anal.calcd. for $C_{17}H_{18}N_4O_4S_2$; M.wt. 406.47. C, 50.23; H, 4.46; N, 13.78.S, 15.78(%); Found: C, 50.21; H, 4.45; N, 13.77; S, 15.77(%).

Ethyl-4-(4-hydroxyphenyl)-6-{[(5-methyl-1,3,4thiadia zol-2-yl)thio]methyl}-2-oxo-1,2,3,4-tetrahydro pyrimi dine-5-carboxylate (3d): Yield (84.5%), w. 0.66g, m.p. 205-207°C pale yellow solid; IR v (cm^{-1}) (KBr): 3368(N-H) , 3117(C-H), 2973 (methyl C-H), 1693 (C=O), 1307 (C-O) ; ^{1}H NMR (400MHz); (DMSO-d_6): δ 9.36 (s, 1H); 9.26 (s, 1H); 7.74(s, 1H); 6.64-7.04 (m, 4H) ArH; 5.17 (d, 1H, J = 4Hz) CH; 4.4-

4.54 (dd, 2H) SCH_2; 3.99 (q, 2H, J= 8Hz) CH_2; 2.69 (s, 3H) CH_3; 1.06 (t, 3H, J =8Hz) CH3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.9, 164.6, 163.4, 156.7, 151.8, 145.4, 134.5, 127.4, 115.0, 102.2, 59.7, 53.4, 33.1, 15.2,13.8., MS *m/z:* 406 (M^+), 407$(M+H)^+$. Anal.calcd. for $C_{17}H_{18}N_4O_4S_2$; M.wt. 406.47. C, 50.23; H, 4.46; N, 13.78.S, 15.78(%) ; Found: C, 50.22; H, 4.45; N, 13.77; S, 15.77(%).

6-(5-methyl-[1,3,4]thiadiazole-2-ylsulfanylmethyl)-2-oxo-4-(3,4,5-trimethoxy-phenyl)-1,2,,3,4-tetrahydro pyrimidine-5-carboxylic acid ethyl ester (3e): Yield (79.5%),w. 0.73g, m.p. 193-195°C pale yellow solid; IR v (cm^{-1}) (KBr): 3315(N-H) , 3270(C-H), 1702(C=O), 1303(C-O) ; ^1H NMR (400MHz); (DMSO-d_6): δ 9.24 (s, 1H) ; 7.79(s, 1H); 6.55 (s, 2H); 5.13 (s, 1H); 4.4-4.59 (q, 2H); 4.02 (d, 2H); 3.70 (s, 6H); 3.34(s, 3H); 2.69 (s, 3H) CH_3;1.10 (t, 3H, J =8Hz) CH_3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.9, 164.6, 152.8, 151.8, 146.7, 139.5, 136.9, 103.5, 101.0, 59.9, 59.8, 55.7, 53.8, 33.3, 15.2, 13.9., MS *m/z:* 480 (M^+), 481$(M+H)^+$. Anal.calcd. for $C_{20}H_{24}N_4O_6S_2$; M.wt. 480.55. C, 49.99; H, 5.03; N, 11.66 ; S, 13.34 (%); Found: C, 49.97; H, 5.02; N, 11.65; S, 13.33(%).

Ethyl6-{[(5-methyl-1,3,4-thiadiazol-2-yl)thio] methyl} -2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carb oxylate (3f): Yield (83.5%), w. 0.63g, m.p. 163-165°C yellow solid; IR v (cm^{-1}) (KBr): 3366 (N-H) ,3091(C-H), 2966 (methyl C-H), 1686 (C=O), 1299(C-O) ; ^1H NMR (400MHz); (DMSO-d_6,): δ 9.36 (s, 1H); 7.88(s, 1H); 7.22-7.30 (m, 5H); 5.17 (d, 1H); 4.40-4.54(dd, 2H); 3.94-4.0(q, 2H); 2.69 (s, 3H) CH_3; 1.08 (t, 3H, J= 8Hz) CH_3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.9, 164.5, 163.4, 151.8, 146.0, 144.0, 128.4, 127.5, 126.2, 101.7, 59.8, 53.9, 33.1, 15.2,13.8., MS *m/z* : 390 (M^+), 391 $(M+H)^+$. Anal.calcd. for $C_{17}H_{18}N_4O_3S_2$; M.wt. 390.47. C, 52.29; H, 4.65; N, 14.35; S, 16.42 (%); Found: C, 52.27; H, 4.64; N, 14.34; S, 16.41(%).

Ethyl-4-(3,4-dihydroxyphenyl)-6-{[(5-methyl-1,3,4thi adiazol-2-yl)thio]methyl}-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (3g): Yield (78%), w. 0.63g, m.p. 198-200°C pale yellow solid. IR v (cm^{-1}) (KBr): 3366 (N-H) ,3108 (C-H), 2969 (methyl C-H), 1689 (C=O), 1290 (C-O); ^1H NMR (400MHz); (DMSO-d_6,): δ 9.69 (s, 1H); 8.83-8.87(2H); 7.71(s, 1H); 6.48-6.67 (m, 3H); 5.17 (d, 1H); 4.40-4.54(dd, 2H); 3.94-4.0(q, 2H); 2.69 (s, 3H) CH_3; 1.08 (t, 3H, J= 8Hz) CH_3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 172.1, 169.9, 168.7, 157.2, 150.2, 150.1, 149.9, 140.3, 122.3, 120.4, 119.0, 107.8, 58.8, 38.3, 20.4, 19.1., MS *m/z* : 422 (M^+), 423 $(M+H)^+$. Anal.calcd. for $C_{17}H_{18}N_4O_5S_2$; M.wt. 422.47. C, 48.33; H, 4.29; N, 13.26 ; S, 15.18 (%); Found: C, 48.31; H, 4.28; N, 13.25; S, 15.17(%).

Ethyl-4-(2-bromo-5-hydroxy-4-methoxyphenyl)-6-{[(5-methyl-1, 3, 4-thiadiazol-2-yl)thio]methyl}-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3h): Yield (80%), w. 0.79g, m.p. 213-215°C brown solid. IR v (cm^{-1}) (KBr): 3351(N-H), 1690 (C=O), 1305 (C-O) ; ^1H NMR (400MHz); (DMSO-d_6): δ 9.19 (s, 1H); 7.72(s, 1H); 7.04(s, 1H); 6.55-6.59 (m, 2H); 5.02 (d, 1H); 4.26-4.52(dd, 2H); 3.89-3.94(q, 2H); 2.47 (s, 3H); 2.43 (s, 3H) CH_3; 1.04 (t, 3H, J= 8Hz) CH_3. ^{13}C NMR (100 MHz); (DMSO-d_6): δ 166.8, 164.3, 163.6, 151.0, 148.1, 146.4, 146.0, 134.6, 115.5, 115.0, 110.1, 101.3, 59.7, 55.9, 53.76, 32.9, 15.2, 13.8., MS *m/z* : 515 $(M)^+$, 517 $(M+H)^{2+}$. Anal.calcd. for $C_{18}H_{19}BrN_4O_5S_2$; M.wt. 515.40. C, 41.95; H, 3.72; N, 10.87 ; S, 12.0 (%); Found: C, 41.93; H, 3.71; N, 10.86; S, 11.99(%).

Antimicrobial activity

Antibacterial activity: The synthesized 4-phenyl-6-(5-methyl-[1,3,4]thiadiazol-2-ylsulfanylmethyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester derivatives were screened for their *in vitro* antibacterial activity against Gram positive (*B. subtilis* (ATCC 6051) and *S. aureus* (ATCC 9144) and Gram negative (*E. coli* (ATCC 25922) and *K. pneumonia* (ATCC 13833) bacterial strains using drug Streptomycin as positive reference compound (10µg) by disc diffusion technique (Thanh *et al*., 2012). All the test compounds were taken in the concentration of 1000 µg and 2000 µg /disc dissolved in DMSO. The target microorganisms were cultured in Mueller Hinton broth (MHB). After 24 h of incubation, the suspensions were adjusted to standard sub-culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strains. Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then, the prepared discs were placed on the culture medium. Then, the inoculated plates were incubated at 37ºC for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-bacterial activity (Table 2).

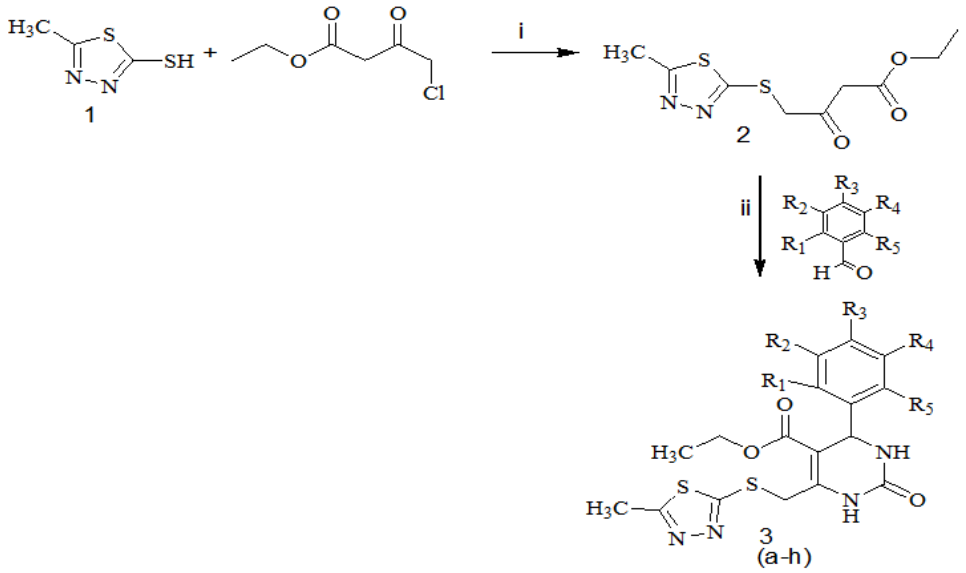
Antifungal activity: All the synthesized titled compounds were screened for antifungal activity by disc diffusion technique (Thanh *et al*., 2012) against *Candida albicans* (MTCC227) and *Aspergillus niger* (MTCC281) using Fluconazole and Clotrimazole as positive reference drug. Potato Dextrose Agar (PDA) medium was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Potato Dextrose Broth (PDB). The synthesized compounds were applied on sterile disc. Standard antibiotic (Fluconazole 15µg and Clotrimazole 15µg) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition around the disc was measured and expressed in millimeters as its anti-fungal activity (Table 3).

Docking studies: The docking studies for tested ligands were performed using molecular modeling software autodock 4.2.6, installed on a single machine running on a 3.3 GHz Intel (R) core (TM) processor with windows 7 as the operating system (Morris *et al*., 2009). Target protein Glucosamine-6-phosphate synthase (PDB ID: 2VF5) used for the docking studies was retrieved from the protein data bank (Mouilleron *et al*., 2008). Protein 2VF5 was refined by removal of water molecules, by adding polar hydrogens and kollmann charges. For the docking studies, a grids box of 44, 50 and 44 points was generated in X, Y and Z axis, respectively, in such way that it covered all residues actively interacting with the co-crystallized ligand. Docking software AutoDock 4.2 Program supplied with AutoGrid 4.0 was used to produce grid maps. ChemBio3D Ultra (version 12) was used to generate the energy minimized conformations of the all ligands in PDB format. Energy minimized conformation of ligands was subjected to calculation of Gasteiger Huckel charges and saved in default format of Autodock. Autodock was run to find 50 possible binding conformations i.e., 50 runs for each docking using LGA search. Default protocol was applied with initial

population of 150 randomly placed individuals, a maximum number of 2.5×105 energy evaluations and 2.7×104 generations. A mutation rate of 0.02 and crossover rate of 0.8 were used.

RESULTS AND DISCUSSION

A series of 2-oxo-6-(5-methyl-[1, 3, 4]-thiadiazol-2-yl sulfanylmethyl)-1, 2, 3, 4-tetrahydropyrimidine compounds were synthesized by two steps. First step of the reaction involved the reaction between 5-methyl-[1, 3,4]thiadiazole-2-thiol (1) and ethyl-4-chloroacetocetate in the presence of anhydrous potassium carbonate in dimethylformamide to form 4-(5-Methyl-[1,3,4]thiadiazol-2-ylsulfanyl)-3-oxo-butyric acid ethyl ester (2) (Mohamed *et al*., 2009). The reaction was monitored by Thin layer chromatography (TLC) using a mixture of ethyl acetate and n-hexane (3:7) as eluent.



Scheme 1. Synthetic route followed for the synthesis of titled compounds 3(a-h)

Table 1. Details of the synthesized compounds 3(a-h)

3	R1	R2	R3	R4	R5
a	H	H	F	H	H
b	Cl	H	H	H	H
c	H	OH	H	H	H
d	H	H	OH	H	H
e	H	OCH3	OCH3	OCH3	H
f	H	H	H	H	H
g	H	OH	OH	H	H
h	Br	H	OCH3	OH	H

The obtained compound was confirmed by mass spectroscopy and used for next step without purification. Subsequent reaction of compound (2) with various benzaldehydes derivatives and urea in presence of hydrochloric acid in ethanol under reflux for 4-6 hr afforded final products (scheme I). So, eight compounds of 4-phenyl-6-(5-methyl-[1,3,4]thiadiazol-2-ylsulfanylmethyl)-2-oxo-1,2,3,4-tetrahydro -pyrimidine-5-carboxylic acid ethyl ester (Table 1) were synthesized by Biginelli reaction (Biginelli *et al*., 1893 ; Yonghong *et al*., 2015 ; Ali *et al*., 2015 ; Fabio *et al*., 2001 ; Mohammad Haji *et al*., 2016 ; Mohammad Hosein Farjam *et al*., 2016). Absorption band at 3364 (-NH str.), 3105 (-CH str), 1688 cm-1(C=O) in IR spectrum and δ7.86 singlet for –NH in ^{1}H NMR spectrum confirmed the structure. Representative ^{1}H-NMR, ^{13}C-NMR, IR and Mass spctras of 3a compound represented in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

Antimicrobial activities evaluation of the compounds 3(a-h)

Antibacterial activity

The synthesized 4-phenyl-6-(5-methyl-[1,3,4]thiadiazol-2-ylsulfanylmethyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester derivatives were screened for their *in vitro* antibacterial activity against Gram positive (*B. subtilis* (ATCC 6051) and *S. aureus* (ATCC 9144) and Gram negative (*E. coli* (ATCC 25922) and *K. pneumonia* (ATCC 13833)) bacterial strains by disc diffusion technique(Thanh-Dao Tran., 2012). The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-bacterial activity (Table 2)

Antifungal activity: All the synthesized titled compounds were screened for antifungal activity by disc diffusion technique (Thanh-Dao Tran., 2012) against *Candida albicans* (MTCC227) and *Aspergillus niger* (MTCC281) using Fluconazole and Clotrimazole as positive reference drug.

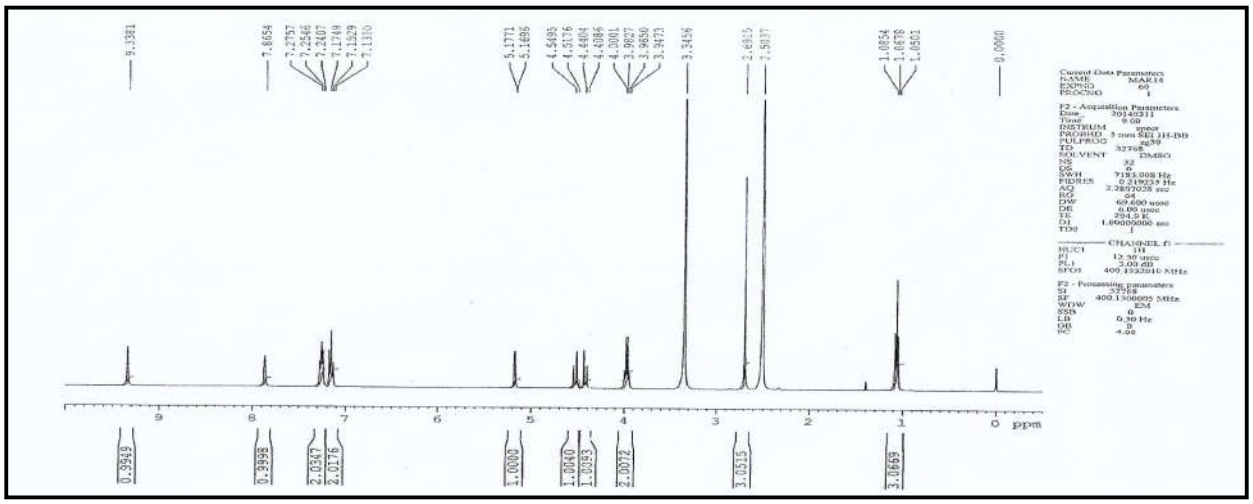


Fig. 1. ^{1}H-NMR spectra of 3a

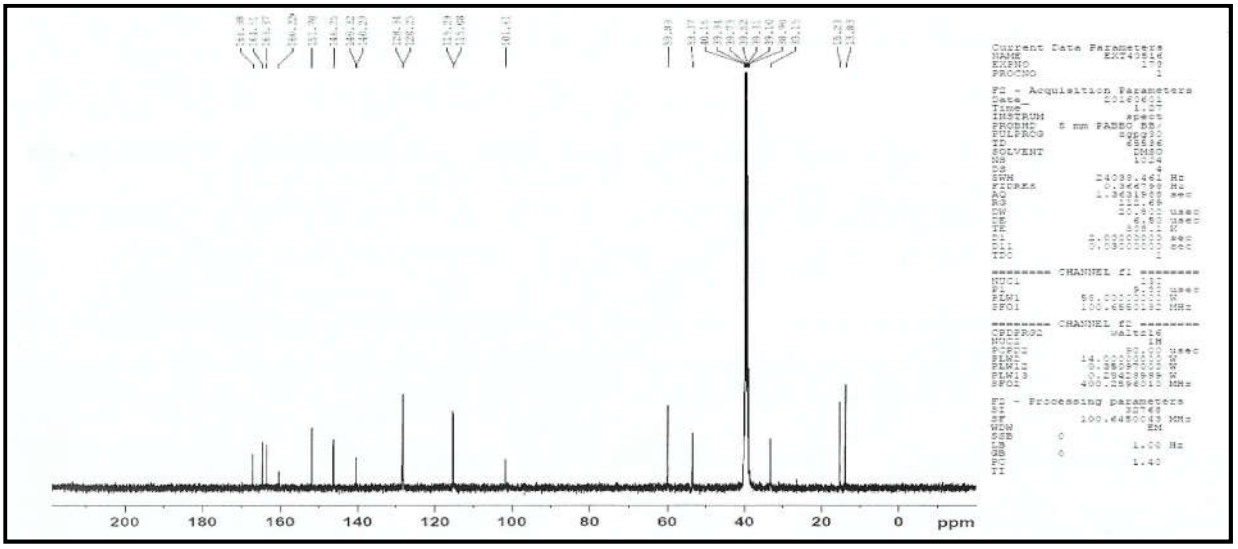


Fig. 2 ^{13}C-NMR spectra of 3a

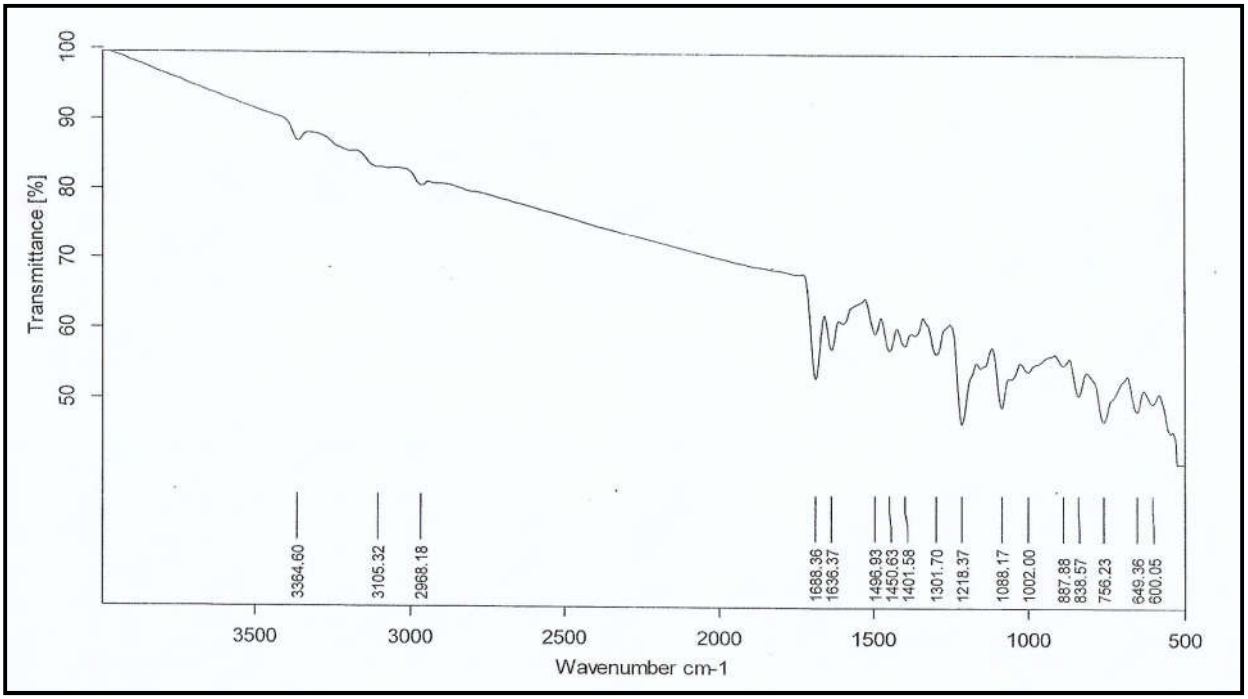


Fig. 3. IR spectra of 3a

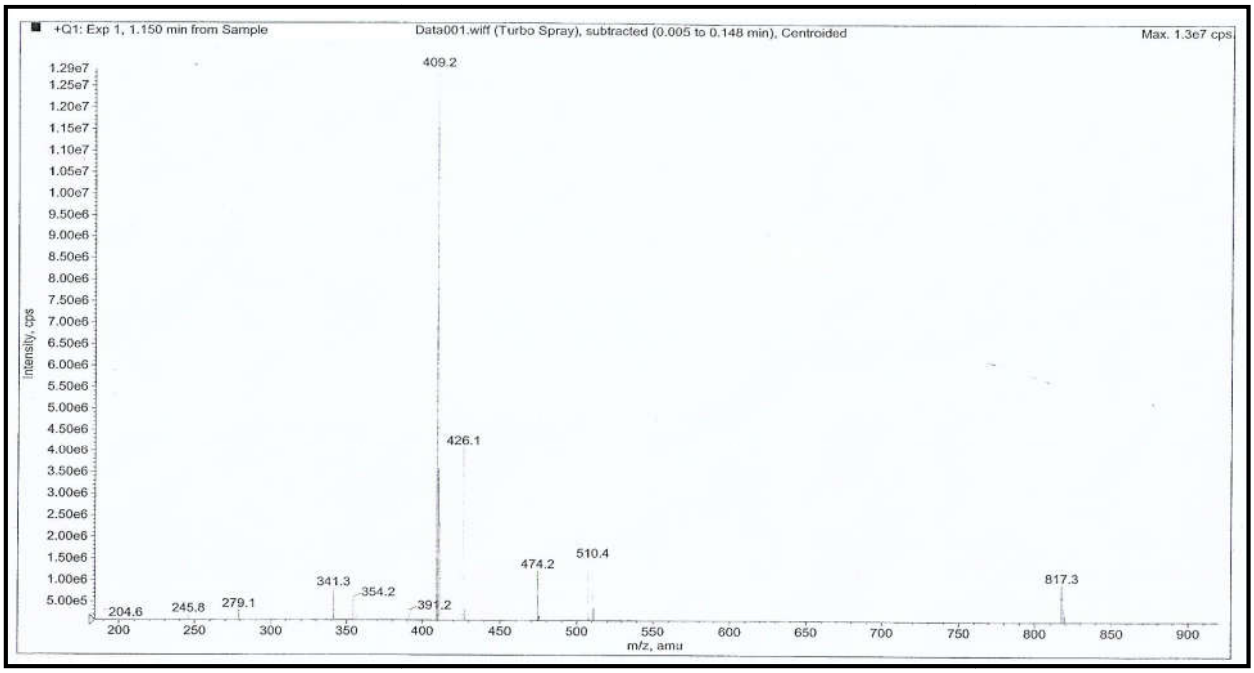


Fig. 4. Mass spectra of 3a

Table 2. Antibacterial activity of synthesized compounds (3a-h)

Compound No	Zone of Inhibition (mm)							
	Gram positive				*Gram negative*			
	S. aereus		*B. subtilis*		*E. coli*		*K. pneumonia*	
	1000µg	2000 µg	1000µg	2000 µg	1000µg	2000 µg	1000µg	2000 µg
3a	12	14	12	15	11	15	-	-
3b	8	13	11	15	0	12	-	-
3c	10	15	13	17	10	13	-	-
3d	11	15	14	17	9	11	-	-
3e	10	14	14	16	8	13	-	8
3f	-	-	10	12	7	14	-	-
3g	9	12	13	16	11	14	-	-
3h	8	11	12	17	7	12	-	-
Streptomycin (10 µg)	24		24		24		22	

- Not active

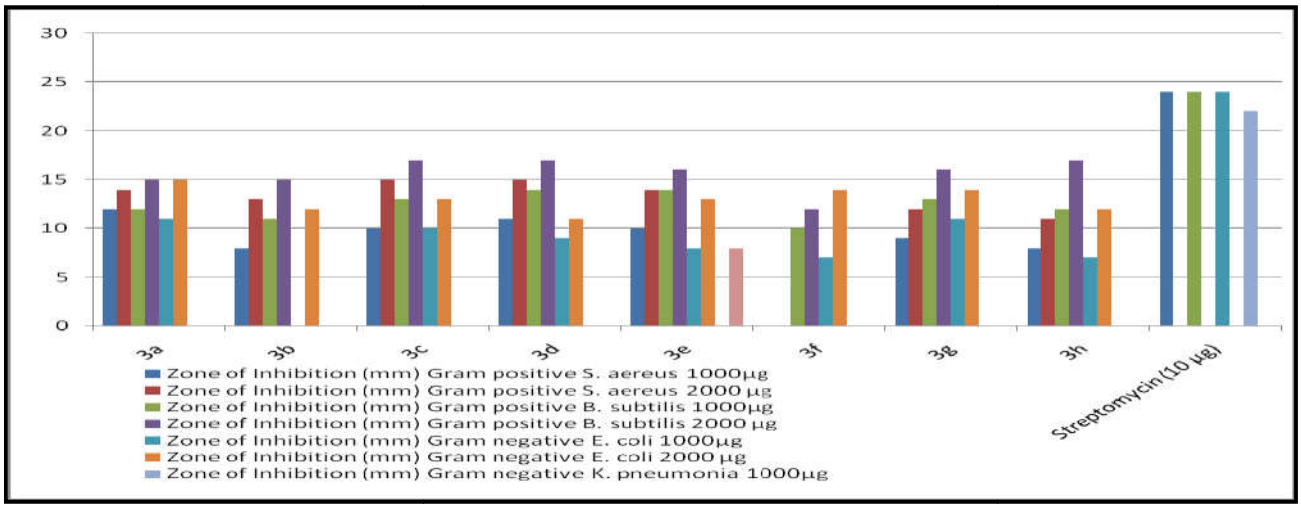


Fig. 5. Bar Diagram for Antibacterial Study for the Synthesised Compounds 3(a-h)

The diameters of zone of inhibition around the disc was measured and expressed in millimeters as its anti-fungal activity (Table 3). The newly synthesized compounds 3(a-h) exhibited mild to moderate antibacterial activity against the tested microorganisms. Compounds 3c, 3d,and 3h showed significant antibacterial activity when compared to standard drug Streptomycin and 3a, 3c, 3e 3f anf 3g showed antifungal activity when compared to standard drug Fluconazole and clotrimazole. From the above discussion made, following SAR can be derived, Substitution on the aromatic ring with halogen substituted and hydroxyl group has a prominent effect on antimicrobial activity. Compounds 3a and 3b having substitution with halogen and 3c and 3b having substitution hydroxyl group exhibited significant activity.

Table 3. Antifungal activity of synthesized compounds (3a-h)

Compound No	Zone of Inhibition (mm)			
	C.albicans		*A.niger*	
	1000µg	2000 µg	1000µg	2000 µg
3a	13	17	7	10
3b	11	14	10	14
3c	12	16	11	17
3d	10	15	9	12
3e	15	20	17	21
3f	14	18	14	18
3g	15	19	16	20
3h	9	14	-	-
Fluconazole (15 µg)	-		24	
Clotrimazole (15 µg)	23		-	

- Not active

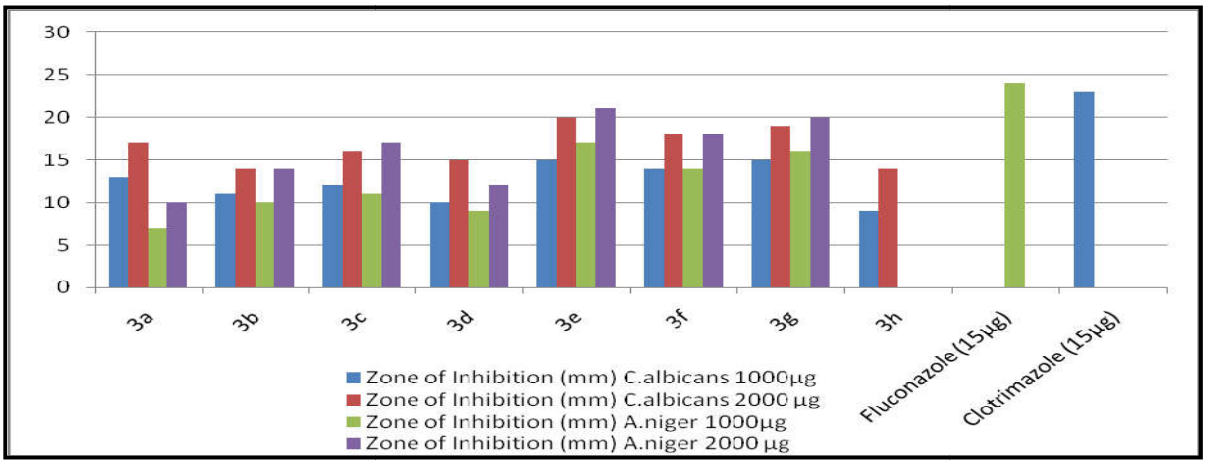


Fig. 6. Bar Diagram for Antifungal study for the Synthesised Compounds 3(a-h)

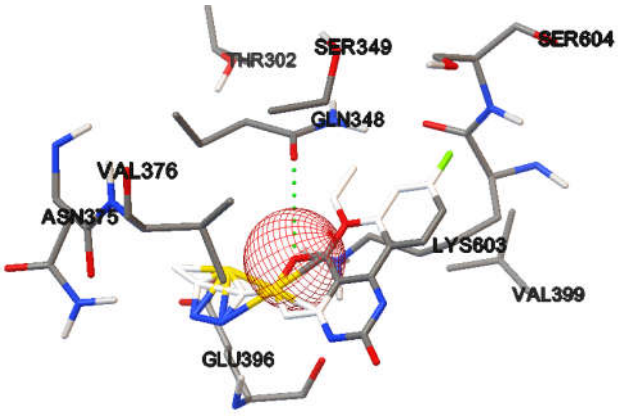


Fig. 7. Docked pose of compound 3a at the active site of 2VF5, showing hydrogen bonding interactions (green dotted lines) with residues Gln-348

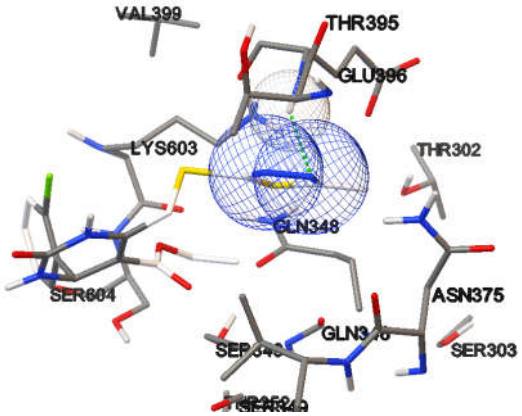


Fig. 8. Docked pose of compound 3b at the active site of 2VF5, showing hydrogen bonding interactions (green dotted lines) with residues Gln-348

Table 4. Docking results of the titled compounds 3(a-h)

Compound No	Docking Score (kcal/mol)	Ki at T = 298.15 K
3a	-13.12	243.41 pM
3b	-12.47	721.82 pM
3c	-11.12	7.07 nM
3d	-10.68	14.81 nM
3e	-9.85	59.82 nM
3f	-11.05	7.93 nM
3g	-10.25	30.86 nM
3h	-9.96	49.70 nM
Reference compound	-8.73	0.39 µM

Substitution on the aromatic ring with hydroxy group has a prominent effect on antifungal activity. Compounds having hydroxy substitution like 3c and 3d displayed significant activity. Compounds having substitution with fluorine and chlorine displayed mild activity.

Docking studies

Docking is a rational drug design approach that seeks to predict binding mode as well as binding free energy of ligand-receptor complex. It not only gives an idea about how ligands

bind with the receptor but also give information about the conformational changes taking place in the receptor structure upon binding with ligand. A study reported by Vijesh *et al*., 2013 revealed that, anti-microbial activity of compounds containing imidazole as central ring. Further the study proposed that reported compounds possessed inhibitory activity due to inhibition of Glucosamine-6-phosphate synthase enzyme which was supported via docking studies on PDB ID: 2VF5. Our reported compounds also possessed thiazole ring with overall pharmacophoric similarity with compounds reported by the former group. Hence, in the current study, the same 2VF5 protein has been selected for the docking studies. Furthermore, for the validation of docking studies, best scoring ligand reported by Vijesh *et al*., 2013 was re-docked according to our protocol and its dock score and interaction pattern were analyzed (Vijesh *et al*., 2013). Re-docking studies of reference compounds (Vijesh *et al*., 2013) revealed that titled compound exhibited docking score -8.73 and inhibition constant (Ki) 0.39 µM which were found in close agreement reported by the former research group (-8.01 and 1.35 µM, respectively). So, based upon the validation studies, we concluded that docking studies could be relied on for the further studies. The result of docking studies in terms of docking score and enzyme inhibition constant value is given in Table 4. The result of the docking studies revealed that compounds showed moderate to significant binding affinity with enzyme with docking score ranging from -9.96 to -13.12 and Ki value 49.70 nM to 243.41pM.

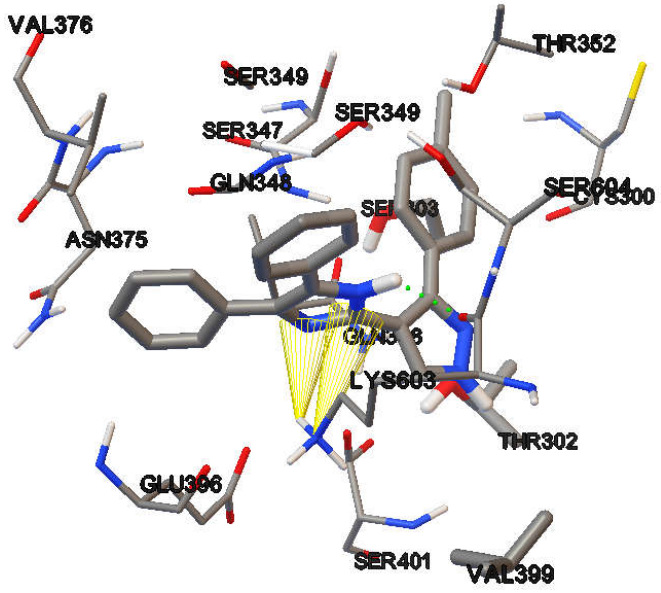


Fig. 9. Docked pose of reference compound at the active site of 2VF5, showing hydrogen bonding (green dotted lines) and pi-cationic interactions (yellow) with Lys-603

Further, it is worthy to note that compound like 3a and 3b showed relatively better antibacterial activity during the in vitro studies; in a similar fashion it also showed best binding affinity during the in silico studies. Best scoring docked poses of compounds 3a and 3b and reference compound inside the active site of 2VF5 enzyme are shown in Figures 7 and 8 respectively.

Conclusion

Present study described the synthesis of novel 2-oxo-6-(5-methyl-[1, 3, 4] thiadiazol-2-ylsulfanylmethyl)-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester derivatives. All the synthesized compounds were characterized by Elemental, Mass, IR, 1H-NMR and 13C NMR spectra and evaluated for antimicrobial activity. The results indicated that, compounds 3c and 3d showed moderate to significant activity against the tested bacterial strains while compounds 3c, 3e, 3f, 3g exhibited significant antifungal activity, while rest of the compounds possessed weak to moderate antimicrobial activity.

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